

What is claimed is:

1. An isolated chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising an amadoriase.

2. The isolated chimeric protein of claim 1, wherein the bacterial leader sequence is a leader sequence of an *E.coli*. protein.

3. The isolated chimeric protein of claim 1, wherein the leader sequence has at least 40% identity to the amino acid sequence set forth in SEQ ID NO:1 (MGGSGDDDDLAL), in which the percentage identity is determined over an amino acid sequence of identical size to the amino acid sequence set forth in SEQ ID NO:1.

4. The isolated chimeric protein of claim 1, wherein the leader sequence binds to an antibody that specifically binds to an amino acid sequence set forth in SEQ ID NO:1.

5. The isolated chimeric protein of claim 1, wherein the leader sequence comprises the amino acid sequence set forth in SEQ ID NO:1.

6. The isolated chimeric protein of claim 1, wherein the first peptidyl fragment comprises about 20 amino acid residues.

7. The isolated chimeric protein of claim 1, wherein the amadoriase is of *Aspergillus sp.* origin.

8. The isolated chimeric protein of claim 7, wherein the amadoriase uses FAD as a cofactor.

9. The isolated chimeric protein of claim 8, wherein the amadoriase has a FAD cofactor-binding consensus sequence Gly-X-Gly-X-X-Gly (SEQ ID NO:2), X being any amino acid residue.

10. The isolated chimeric protein of claim 7, wherein the amadoriase is selected from the group consisting of amadoriase Ia, amadoriase Ib, amadoriase Ic and amadoriase II.

11. The isolated chimeric protein of claim 1, wherein the amadoriase has at least 40% identity to the amino acid sequence set forth in SEQ ID NO:3 (AVTKSSSLIVGAGTWGTSTALHLARRGYTNVTVLDPYPVPSAISAGNDV NKVISSGQYSNNKDEIEVNEILAEAAFNGWKNDPLFKPYYHDTGLLMSAC SQEGLDRLGVRVRPGEDPNLVELTRPEQFRKLAPEGVLQGDFPGWKGYF ARSGAGWAHARNALVAAAREAQRMGVKFVTGTPQGRVVTLIFENNDVK GAVTGDGKIWRAERTFLCAGASAGQFLDFKNQLRPTAWTLVHIALKPEE RALYKNIPVIFNIERGFFFEPPDEERGEIKICDEHPGYTNMVQSADGTMMSIP FEKTQIPKEAETRVRALLKETMPQLADRPFSFARICWCADTANREFLIDRH PQYHSLVLGCGASGRGFKYLPISIGNLIVDAMEGKVPQKIHელიკWNPDIAA NRNWRDTLGRFGGPNRVMDVFHDVKEWTVNVQYRDISKL), in which the percentage identity is determined over an amino acid sequence of identical size to the amino acid sequence set forth in SEQ ID NO:3.

12. The isolated chimeric protein of claim 1, wherein the amadoriase binds to an antibody that specifically binds to an amino acid sequence set forth in SEQ ID NO:3.

13. The isolated chimeric protein of claim 1, wherein the amadoriase comprises the amino acid sequence set forth in SEQ ID NO:3.

14. The isolated chimeric protein of claim 1, wherein the first and second peptidyl fragments are linked via a cleavable linkage.

15. The isolated chimeric protein of claim 1, which further comprises, at its C-terminus, a third peptidyl fragment comprising a second bacterial leader sequence from about 5 to about 30 amino acid residues.

16. The isolated chimeric protein of claim 15, wherein the second bacterial leader sequence is a leader sequence of an *E.coli* protein.

17. The isolated chimeric protein of claim 15, wherein the second bacterial leader sequence has at least 40% identity to the amino acid sequence set forth in SEQ ID NO:4 (KGELEGLPIPPLLRTG), in which the percentage identity is determined over an amino acid sequence of identical size to the amino acid sequence set forth in SEQ ID NO:4.

18. The isolated chimeric protein of claim 15, wherein the second bacterial leader sequence binds to an antibody that specifically binds to an amino acid sequence set forth in SEQ ID NO:4.

19. The isolated chimeric protein of claim 15, wherein the second bacterial leader sequence comprises the amino acid sequence set forth in SEQ ID NO:4.

20. The isolated chimeric protein of claim 15, wherein the third peptidyl fragment comprises about 20 amino acid residues.

21. The isolated chimeric protein of claim 1, which further comprises, at its C-terminus, a third peptidyl fragment comprising a peptide tag.

22. The isolated chimeric protein of claim 21, wherein the peptide tag is selected from the group consisting of FLAG, HA, HA1, c-Myc, 6-His, AU1, EE, T7, 4A6, ϵ , B, gE and Ty1 tag.

23. The isolated chimeric protein of claim 15, which further comprises, at its C-terminus, a fourth peptidyl fragment comprising a peptide tag.

24. The isolated chimeric protein of claim 23, wherein the peptide tag is selected from the group consisting of FLAG, HA, HA1, c-Myc, 6-His, AU1, EE, T7, 4A6, ϵ , B, gE and Ty1 tag.

25. The isolated chimeric protein of claim 1, which comprises the amino acid sequence set forth in SEQ ID NO:5
(MGGSGDDDDLALAVTKSSSLIVGAGTWGTSTALHLARRGYTNVTVLD
PYPVPSAISAGNDVNKVISSGQYSNNKDEIEVNEILAEAFNGWKNDPLFK
PYYHDTGLLMSACSQEGLDRLGVRVRPGEDPNLVELTRPEQFRKLAPEGV
LQGDFFPGWKGYFARSGAGWAHARNALVAAAREAQRMGVKFVTGTPQG
RVVTLIFENNDVKGAVTGDGKIWRAERTFLCAGASAGQFLDFKNQLRPT
AWTLVHIALKPEERALYKNIPVIFNIERGFFFEPEERGEIKICDEHPGYTN
MVQSADGTMMSIPFEKTQIPKEAETRVRALLKETMPQLADRPFSFARICW
CADTANREFLIDRHPQYHSLVLGCGASGRGFKYLPSIGNLIVDAMEGKVP
QKIHელიKWNPDIAANRNWRDTLGRFGGPNRVMDFDVKEWTNVQYRDI
SKLKGELEGLPIPNPLLRTGHHHHHH).

26. An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 1.

27. An isolated nucleic acid comprising a nucleotide sequence encoding the chimeric protein of claim 25.

28. The nucleic acid of claim 26, which comprises the nucleotide sequence set forth in SEQ ID NO:6

(ATGGGAGGTTCTGGGTGACGATGATGACCTGGCTCTCGCCGTCCTAA
GTCATCATCTCTCCTGATCGTTGGTGCCGGGACTTGGGGCACCTCAAC
GGCTCTGCACCTCGCGCGCCGCGGATATACCAACGTTACCGTGCTGGA
CCCCTATCCTGTCCCTAGCGCCATCTCCGCCGGAACGACGTGAACAA
AGTCATTAGCAGTGGCCAATATTCGAATAACAAAGACGAAATCGAAG
TGAATGAGATCTTGGCGGAAGAGGCGTTTAACGGTTGGAAGAACGAC
CCGCTTTTCAAACCGTATTATCATGATACGGGCCTGCTGATGTCTGCTT
GCTCGCAGGAGGGCCTGGATCGCCTGGGCGTCCGGGTACGTCCGGGCG
AGGATCCTAATCTGGTGGAACTTACCCGCCCGGAGCAATTTTCGTAAAC
TGGCCCCGGAAGGCGTGTTGCAAGGTGATTTTCCGGGTGGAAGGGT
ACTTTGCGCGTTCCGGCGCTGGCTGGGCACATGCAAGGAATGCCTTAG
TGGCAGCAGCACGCGAAGCACAGCGCATGGGTGTAAAATTTGTTACTG
GCACCCCGCAGGGTCGTGTAGTCACGTTAATCTTTGAAAATAACGATG
TAAAAGGTGCCGTTACGGGCGATGGCAAAATTTGGAGAGCGGAACGT
ACATTCCTGTGTGCTGGGGCTAGCGCGGGTCAGTTCCTAGATTTCAAG
AATCAACTTCGACCAACCGCTTGGACCCTGGTACACATTGCGTTAAAA
CCGGAAGAACGTGCGTTGTACAAAAATATACCGGTTATCTTTAACATC
GAACGGGGGTTTTTCTTTGAACCCGATGAGGAGCGCGGTGAGATTAAA
ATATGCGATGAACACCCGGGCTACACAAATATGGTCCAGAGTGCAGA
CGGCACGATGATGAGCATTCCGTTTCGAAAAAACCCAGATTCCAAAAG
AAGCCGAAACGCGCGTTCCGGGCCCTGCTGAAAGAGACAATGCCCCAG
CTGGCAGACCGTCCATTTCAGCTTCGCACGCATTTGCTGGTGTGCCGAT
ACCGCGAATCGCGAATTCCTGATAGATCGACATCCGCAGTACCACAGT
CTTGTGTTGGGCTGTGGTGCGAGCGGAAGAGGGTTTAAATATCTGCCT
TCTATTGGGAATCTCATTGTTGACGCGATGGAAGGTAAAGTGCCGCAA
AAAATTCACGAATTAATCAAGTGGAAACCCGGACATTGCGGCGAACCGT
AACTGGCGTGATACTCTGGGGCGTTTTGGCGGTCCAAATCGTGTGATG
GATTTTCATGATGTGAAGGAATGGACCAATGTTTCAGTATCGTGATATT

TCCAAGCTGAAAGGAGAGTTGGAAGGTaaGCCAATCCCTAACCCGTTA
CTGCGCACAGGCCATCACCATCATCATATTAA).

29. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 26.

30. A recombinant cell containing the nucleic acid of claim 26.

31. A method of producing a chimeric protein comprising growing a recombinant cell containing the nucleic acid of claim 26 such that the encoded chimeric protein is expressed by the cell, and recovering the expressed chimeric protein.

32. The product of the method of claim 31.

33. A method for assaying for a glycosylated protein in a sample, which method comprises:

- a) contacting a sample to be assayed with a protease to generate a glycosylated peptide or a glycosylated amino acid from a glycosylated protein, if contained in said sample;
- b) contacting said generated glycosylated peptide or glycosylated amino acid with a chimeric protein of claim 1 to oxidize said glycosylated peptide or glycosylated amino acid; and
- c) assessing oxidation of said glycosylated peptide or glycosylated amino acid by said chimeric protein to determine the presence and/or amount of said glycosylated protein in said sample.

34. The method of claim 33, wherein the sample is a blood sample.

35. The method of claim 34, wherein the blood sample is a plasma, serum, red blood cell or whole blood sample.

36. The method of claim 33, wherein the glycated protein to be assayed is glycoalbumin or glycohemoglobin.

37. The method of claim 33, wherein the protease is an endo-type protease or an exo-type protease.

38. The method of claim 37, wherein the endo-type protease is selected from the group consisting of trypsin, α -chymotrypsin, subtilisin, proteinase K, papain, cathepsin B, pepsin, thermolysin, protease XVII, protease XXI, lysyl-endopeptidase, prolether and bromelain F.

39. The method of claim 37, wherein the exo-type protease is an aminopeptidase or a carboxypeptidase.

40. The method of claim 33, wherein the protease is selected from the group consisting of proteinase K, pronase E, ananase, thermolysin, subtilisin and cow pancreas proteases.

41. The method of claim 33, wherein the protease generates a glycated peptide from about 2 to about 30 amino acid residues.

42. The method of claim 33, wherein the protease generates glycated glycine, glycated valine or glycated lysine residue or a glycated peptide comprising glycated glycine, glycated valine or glycated lysine residue.

43. The method of claim 33, wherein the chimeric protein comprises the amino acid sequence set forth in SEQ ID NO:5.

44. The method of claim 33, wherein the chimeric protein is encoded by the nucleotide sequence set forth in SEQ ID NO:6.

45. The method of claim 33, wherein the oxidation of the glycated peptide or glycated amino acid is assessed by assessing consumption of the glycated peptide or glycated amino acid, H_2O or O_2 in the oxidation reaction or the formation of the oxidized glucose (glucosone), H_2O_2 or the amino acid in the oxidation reaction.

46. The method of claim 45, wherein the O_2 consumption is assessed by an oxygen electrode.

47. The method of claim 45, wherein the H_2O_2 formation is assessed by a peroxidase.

48. The method of claim 47, wherein the peroxidase is horseradish peroxidase.

49. The method of claim 47, wherein the H_2O_2 formation is assessed by a peroxidase and Trinder reaction.

50. The method of claim 47, wherein the glycated peptide or glycated amino acid is contacted with the chimeric protein and the peroxidase sequentially or simultaneously.

51. The method of claim 45, wherein the glucosone formation is assessed by a glucose oxidase.

52. The method of claim 45, wherein the glucosone formation is assessed by a combination of glucose 6-phosphate dehydrogenase and hexokinase.

53. The method of claim 33, wherein the protease is inactivated before or current with the contact between the glycated peptide or glycated amino acid and the chimeric protein.

54. The method of claim 53, wherein the protease is inactivated by a heat treatment or an inhibitor of the protease.

55. *The method of claim 33, wherein ascorbate interference is countered using a copper (II) compound, a cholic acid or a bathophenanthroline disulphonic acid or a mixture thereof.*

56. The method of claim 33, wherein bilirubin interference is countered using a ferrocyanide salt.

57. The method of claim 33, which is used in the prognosis or diagnosis of a disease or disorder.

----- 58. *The method of claim 57, wherein the disease or disorder is diabetes.* -----

59. A kit for assaying for a glycated protein in a sample, which kit comprises:

a) a protease to generate glycated peptide or glycated amino acid from a glycated protein, if contained in a sample;

b) a chimeric protein of claim 1 to oxidize said glycated peptide or glycated amino acid; and

c) means for assessing oxidation of said glycated peptide or glycated amino acid by said chimeric protein to determine the presence and/or amount of said glycated protein in said sample.

60. The kit of claim 59, wherein the means for assessing oxidation of said glycated peptide or glycated amino acid by said chimeric protein comprises peroxidase.

61. The kit of claim 60, wherein the chimeric protein and the peroxidase are formulated in a single composition.

62. A method for assaying for a glycated protein in a sample, which method comprises:

- a) contacting a sample to be assayed with a proteinase K to generate a glycated peptide or a glycated amino acid from a glycated protein, if contained in said sample;
- b) contacting said generated glycated peptide or glycated amino acid with an amadoriase to oxidize said glycated peptide or glycated amino acid; and
- c) assessing oxidation of said glycated peptide or glycated amino acid by said amadoriase to determine the presence and/or amount of said glycated protein in said sample.

63. The method of claim 62, wherein the sample is a blood sample.

64. The method of claim 63, wherein the blood sample is a plasma, serum, red blood cell or whole blood sample.

65. The method of claim 62, wherein the glycated protein to be assayed is glycoalbumin or glycohemoglobin.

66. The method of claim 62, wherein the amadoriase comprises a chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
- b) a second peptidyl fragment comprising an amadoriase.

67. The method of claim 66, wherein the chimeric protein comprises the amino acid sequence set forth in SEQ ID NO:5.

68. The method of claim 66, wherein the chimeric protein is encoded by the nucleotide sequence set forth in SEQ ID NO:6.

69. The method of claim 62, wherein the oxidation of the glycated peptide or glycated amino acid is assessed by assessing consumption of the glycated peptide or glycated amino acid, H_2O or O_2 in the oxidation reaction or the formation of the oxidized glucose (glucosone), H_2O_2 or the amino acid in the oxidation reaction.

70. The method of claim 69, wherein the O_2 consumption is assessed by an oxygen electrode.

71. The method of claim 69, wherein the H_2O_2 formation is assessed by a peroxidase.

72. The method of claim 71, wherein the peroxidase is horseradish peroxidase.

73. The method of claim 71, wherein the H_2O_2 formation is assessed by a peroxidase and Trinder reaction.

74. The method of claim 71, wherein the glycated peptide or glycated amino acid is contacted with the amadoriase and the peroxidase sequentially or simultaneously.

75. The method of claim 69, wherein the glucosone formation is assessed by a glucose oxidase.

76. The method of claim 69, wherein the glucosone formation is assessed by a combination of glucose 6-phosphate dehydrogenase and hexokinase.

77. The method of claim 62, wherein the proteinase K is inactivated before or current with the contact between the glycated peptide or glycated amino acid and the amadoriase.

78. The method of claim 77, wherein the proteinase K is inactivated by a heat treatment or an inhibitor of the proteinase K.

79. The method of claim 62, wherein ascorbate interference is countered using a copper (II) compound, a cholic acid or a bathophenanthroline disulphonic acid or a mixture thereof.

80. The method of claim 62, wherein bilirubin interference is countered using a ferrocyanide salt.

81. The method of claim 62, which is used in the prognosis or diagnosis of a disease or disorder.

82. The method of claim 81, wherein the disease or disorder is diabetes.

83. A kit for assaying for a glycated protein in a sample, which kit comprises:

- a) a proteinase K to generate a glycated peptide or a glycated amino acid from a glycated protein, if contained in said sample;
 - b) an amadoriase to oxidize said glycated peptide or glycated amino acid;
- and

c) means for assessing oxidation of said glycated peptide or glycated amino acid by said amadoriase to determine the presence and/or amount of said glycated protein in said sample.

84. The kit of claim 83, wherein the means for assessing oxidation of said glycated peptide or glycated amino acid by said amadoriase comprises peroxidase.

85. The kit of claim 84, wherein the amadoriase and the peroxidase are formulated in a single composition.

86. The kit of claim 83, wherein the amadoriase comprises a chimeric protein, which chimeric protein comprises, from N-terminus to C-terminus:

- a) a first peptidyl fragment comprising a bacterial leader sequence from about 5 to about 30 amino acid residues; and
 - b) a second peptidyl fragment comprising an amadoriase.
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